# SCARB1 variants and HCV infection:

# Host susceptibility is lost in translation

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Approximately 150 million people are chronically infected with hepatitis C virus (HCV). Despite the remarkable advancement of direct-acting antivirals (DAAs) in recent years to achieve high cure rates, HCV remains a major global health burden. In the absence of curative therapy, chronically infected individuals are at high risk to develop severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Even viral cure does not eliminate the risk of HCC in all patients [1]. Approximately 25% of HCV-infected individuals spontaneously clear infection in the acute phase and do not go on to become chronically infected [2]. Furthermore, some intravenous drug users who are frequently exposed to HCV over prolonged periods of time appear to be protected from HCV re-infection [3]. These observations highlight that inter-person variability markedly affects the clinical course of HCV infection and liver disease. Indeed, genetic variation in interferon lambda 3 and interferon lambda 4 has been strongly associated with clearance of HCV infection and responsiveness to interferon-based therapies [4, 5]. However, the impact of genetic variation of host-dependency factors for HCV infection is still poorly understood. In this issue of *Journal of Hepatology*, Westhaus and colleagues address this question by examining whether genetic variants of a crucial HCV entry factor, scavenger receptor class B type I (SR-BI), influence HCV replication and clinical outcome [6].

Along with the cluster of differentiation 81 (CD81) and the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN), SR-BI is one of the canonical HCV entry factors [7]. SR-BI is encoded by the *SCARB1* gene, which is located on chromosome 12. SR-BI is a critical receptor for high density lipoproteins (HDL), and interacts with the HCV E2 protein [8] and possibly also virion-associated lipoproteins [9] to mediate an early HCV entry step [10]. Furthermore, SR-BI is a promising antiviral target, as shown

by completed proof-of-concept for anti-SR-BI monoclonal antibodies and a small molecule, which are in preclinical and clinical development, respectively [7]. Genetic variation in the *SCARB1* gene has been associated with clinical phenotypes related to altered serum levels of HDL and other lipoproteins [11, 12]. However, little is known about the impact of these variants on HCV infection. Here, Westhaus et al. comprehensively evaluate coding and non-coding variants of *SCARB1* to show that coding non-synonymous genetic variants do indeed influence HCV replication [6]. Furthermore, the authors identify non-coding variants that may influence the clinical outcome of infection.

The authors first selected five coding non-synonymous (i.e., with altered amino acid sequence) *SCARB1* variants with known clinical phenotypes related to HDL levels: SR-BI G2S, S112F, V135A, T175A and P297S. Using lentiviral transduction, these five variants were expressed in hamster CHO-745 cells (with very low expression of endogenous SR-BI) or SR-BI-deficient Huh7.5 cells generated by CRISPR/Cas9 engineering. All variants were present on the cell surface at similar levels as wild type SR-BI, although S112F and T175A were not able to bind soluble HCV E2 protein. Furthermore, variants S112F and T175A demonstrated impaired cell-free HCV entry (HCVpp) or infection (HCVcc) in NIH3T3/CD81-CLDN1-OCLN triple transduced cells or in SR-BI-knockout Huh7.5 cells. Interestingly, S112F and T175A variants allowed direct cell-to-cell transmission of HCV. Indirect evidence suggested that the lipid transfer abilities of S112F and T175A mutants in Huh7.5 cells were not impaired. However, it should be noted that these mutations were previously described to abrogate both binding to HDL and lipid transfer activity in Huh7 cells [13] and COS-7 cells [14]. Nonetheless,

S112F and T175A were impaired in their ability to function as HCV receptors in both Huh7.5 and Huh7 cells **(Figure 1)** [6, 13]. The most likely possibility, which remains to be tested, is that SR-BI variants S112F and T175A fail to adopt a conformation that supports HCV binding and entry, or have altered glycosylation patterns which impair their function.

Westhaus et al. also investigated the potential impact of non-coding *SCARB1* variants on HCV infection, using four synonymous (i.e., no amino acid sequence change) single-nucleotide polymorphisms (SNPs): rs10846744, rs2278986, rs3782287 and rs5888. These variants are known to produce altered serum lipid profiles, likely by affecting expression of SR-BI [15]. Genotyping and genetic association studies were performed on a population of 262 HCV genotype 1-infected individuals from the INDIV-2 cohort [16]. Interestingly, rs3782287 and rs5888 were associated with viral load, and rs3792287/rs5888 A/C was identified as a risk allele for increased viral load. Furthermore, the rs3782287 G allele was associated with decreased viral load **(Figure 1)**. Westhaus et al. postulated that rs3782287/rs5888 non-coding variants might affect viral load by modulating the expression levels of SR-BI. Indeed, in liver tissue from 40 patients, a higher number of patients in the rs3872287 GG genotype group (associated with decreased viral load) had lower or absent hepatic SR-BI expression compared to other genotype groups, although this finding did not reach statistical significance. Further validation studies in larger patient cohorts will be useful to elucidate the clinical relevance and significance of this finding.

What is the clinical impact of the SR-BI variants described here? For the coding variants, all are rare in human populations (e.g., reported minor allele frequency of <1%

for the T175A variant). The HCV serostatus of individuals with S112F and T175A variants is not known, although it is likely that these variants confer some degree of protection to HCV infection. Similarly, genetic variants of the co-receptor CCR5 for human immunodeficiency virus-1 (HIV-1) do indeed protect individuals from HIV-1 infection [17]. The CCR5-**Δ**32 mutation prevents expression of the truncated protein on the cell surface, thereby precluding its function as an HIV-1 entry factor. In the case of HCV, the recently defined redundancy in HCV receptor function of SR-BI and lipoprotein receptors (low density lipoprotein receptor, LDLR and very low density lipoprotein receptor, VLDLR) [13], suggests that SR-BI coding variants may not confer full protection against HCV entry. However, as lipid transport function appears to be impaired in these variants [13, 14], other stages in HCV replication may also be affected, given the close relationship between lipid metabolism and HCV replication and assembly steps [18]. To address this question it will be important to evaluate the HCV serostatus of individuals with S112F and T175A variants or, conversely, the SR-BI genotype of individuals who clear acute HCV infection or appear resistant to acquiring HCV infection.

The clinical impact of genetic variants of *SCARB1* on HCV infection in different ethnic populations remains to be fully defined. In the predominantly Caucasian cohort evaluated here, the rs3782287 GG genotype was associated with lower viral load, but there was no significant association with response to interferon/ribavirin therapy [6]. In contrast, the rs10846744 GG genotype (but not rs3782287) was associated with lower viral load in an Asian cohort, yet surprisingly was associated with a decreased sustained virological response (SVR) rate and unfavourable therapeutic outcomes in the context of

pegylated interferon plus ribavirin therapy [19]. As these studies evaluated only pegylated interferon/ribavirin therapy, it would be interesting to further evaluate potential association between treatment outcome/kinetics in the era of DAA therapy. Given that these non-coding variants are relatively prevalent in human populations (minor allele frequencies between 25% and 42%), further studies in different populations are warranted to elucidate the relationship between non-coding SR-BI variants, HCV viral load and treatment outcome.

In the context of HIV infection, the CCR5-**Δ**32 allele confers a selective advantage. Therefore, it has been under strong selective pressure throughout its evolutionary history, now existing at a frequency of ~10% in European populations [20]. It would be interesting to evaluate potential evolutionary selection pressures relating to the *SCARB1* genetic variants described herein, especially the non-coding variants reaching minor allele frequencies of up to 42%. Although it is unlikely that HCV infection could in itself account for these evolutionary selective pressures on *SCARB1*, the mechanisms underlying the genetic variation of *SCARB1* could provide further insight into HCV-host interactions and disease biology.

In conclusion, Westhaus and colleagues show that coding and non-coding *SCARB1* variants affect the HCV life cycle by impairing entry and reducing viral load, respectively. This elegant example of genetic variation affecting HCV entry contributes to our understanding of the inter-individual variation during HCV infection, and further underscores both the importance of SR-BI as an HCV host factor and its relevance as an antiviral target.

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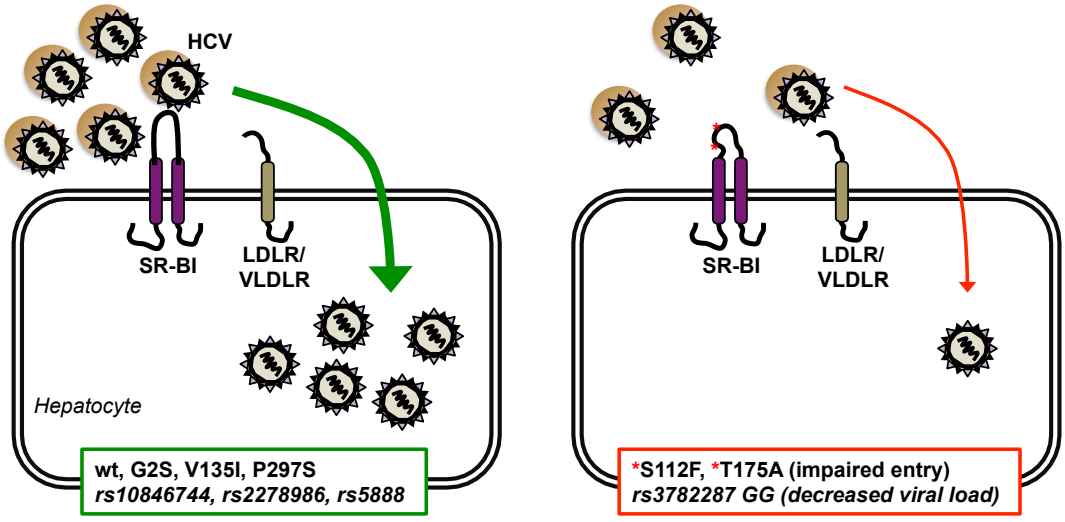


**Figure 1. Impact of SCARB1 variants on HCV infection.** HCV particles bind to SR-B1 during an early entry step, enabling subsequent interactions with other entry factors (not shown for clarity) and productive infection of hepatocytes (left). Certain non-synonymous coding variants and synonymous non-coding variants (italicized) modulate HCV infection (right) [6]. Coding variants S112F and T175A do not function as HCV receptors, likely as a result of conformational changes to SR-BI. The non-coding variant rs3782287 G allele is associated with decreased viral load, possibly due to decreased SR-BI expression. Receptor redundancy may allow a decreased level of HCV entry via other lipoprotein receptors, LDLR and VLDLR [13]. HCV, hepatitis C virus; SR-BI, scavenger receptor class B type I; LDLR, low-density lipoprotein receptor; VLDLR, very low-density lipoprotein receptor.